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Electronic Supplementary Information

DNA sequences: Sequences S1-S4 are identical to those in Goodman et al., *Science* **2005**, *310*, 1661. Sequences R1, M1, and M2 are listed below.
S1: 5'-AGGCAGTTGAGACGAACATTCCTAAGTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC-3'
S2: 5'-CTTGCTACACGATTCAGACTTAGGAATGTTCGACATGCGAGGGTCCAATACCGACGATTACAG-3'
S3: 5'-GGTGATAAAACGTGTAGCAAGCTGTAATCGACGGGAAGAGCATGCCCATCCACTACTATGGCG-3'
S4: 5'-CCTCGCATGACTCAACTGCCTGGTGATACGAGGATGGGCATGCTCTTCCCGACGGTATTGGAC-3'
R1: 5'-GGTGATACGTCTACTATGGCGGGTGATAAATTTCAGACTTAGGAATGTTCGTCTCAACTGCCT-3'
M1: 5'-AGGCAGTTGAGACTAAGCGAACATTCTCTGAAATTTATCACCCGCCATAGTAGACGTATCACC-3'
M2: 5'-CTTGCTACACGATTCAGAGAATGTTCGCTTAGACATGCGAGGGTCCAATACCGACGATTACAG-3'

Assembly of tetrahedra: All DNAs were purchased from Integrated DNA Technology and diluted in TM buffer (10 mM Tris, 5 mM $MgCl_2$) to a final total concentration of 0.8 μM. Solutions were heated at 95 ^{o}C for 5 minutes, followed by rapid cooling to 4 ^{o}C using a thermocycler (Bio-Rad).

Enzymatic digestion and serum stability: All enzymes were purchased from New England Biolabs. In the case of DdeI digestion, 0.4 μM of assembled T1 and T2 DNA tetrahedra were first ligated by T4 DNA ligase overnight, followed by the addition of 10 U of Exo III and incubation for 30 min at 37 ^{o}C. Exo III was inactivated by heating at 70 ^{o}C for 20 min. The mixtures were then re-assembled using a thermocycler, and 0.07 μg of either tetrahedra DNA or linear DNA were incubated with 2 U of DdeI (30.7 molar ratio enzyme:substrate) for 1 h at 37 ^{o}C. For DNase I digestion, 0.2 U of DNase I (0.77 molar ratio enzyme:substrate) was used with incubation at 37 ^{o}C for the indicated times. For serum incubation, 11 uL of fetal bovine serum (ATCC) were added to 100 μL of 0.8 μM solution of tetrahedra and linear DNA, and incubated at 37°C and 5 % CO_2. Each sample was taken after desired time of incubation.

Gel electrophoresis: Solutions of digested mixtures were mixed with sample buffer containing formamide and heated at 75 ^{o}C for 10 min. Subsequently, they were run on a 12% denaturing PAGE containing 8 M urea in 1x TAE buffer. Native gels were synthesized without addition of formamide and urea. For migration of ligated pyramid, a gradient gel of 5 -12 % was utilized. Bands were visualized with Gelstar staining (Lonza) and analyzed using ImageJ software.

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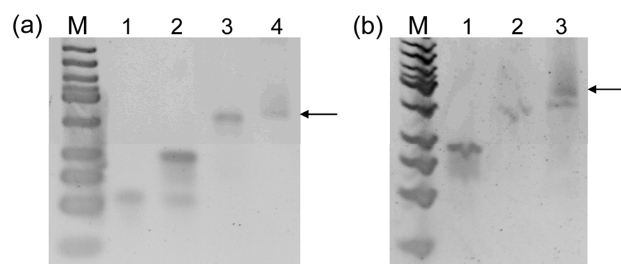

Figure S1. Assembly of tetrahedra T1 (a) and T2 (b). Each component DNA was first heated at 95 °C for 5 minutes and sequentially added to the mixture for hybridization at 42 °C. The resulting structures were analyzed in a 4% native gel. (a) lane 1, S3; lane 2, S3 + S4; lane 3, S3 + S4 + S1; lane 4, S3 + S4 + S1 + S2. (b) lane 1, S3 + S4; lane 2, S3 + S4 + M1; lane 3, S3 + S4 + M1 + M2. The arrows indicate final tetrahedral structures, and we note that T2 is not formed as efficiently as T1.

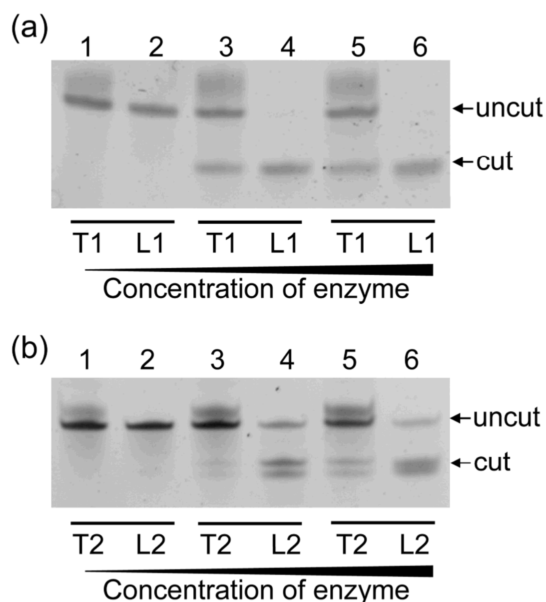

Figure S2. DdeI digestion of unligated tetrahedra T1 (a) and T2 (b). Since each tetrahedron is composed of two DNA strands which contain the DdeI site, and two DNA strands which lack the DdeI site, efficient digestion of DdeI will decrease the intensity of uncut bands by *half*. The generation of shorter DNA fragments from both T1 and T2 indicates that unligated tetrahedra were not able to block DdeI digestion. Lanes 1-2 are uncut controls, lanes 3-4 are digests with 4x excess of enzyme and lanes 5-6 are digests with 40x excess of enzyme.

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